

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number  
**WO 00/58488 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 15/87, 15/62**

(74) Agent: LEARN, June, M.; Gray Cary Ware & Freidenrich LLP, Suite 1600, 4365 Executive Drive, San Diego, CA 92121 (US).

(21) International Application Number: **PCT/US00/08571**

(22) International Filing Date: 31 March 2000 (31.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/127,467 31 March 1999 (31.03.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:  
US 60/127,467 (CIP)  
Filed on 31 March 1999 (31.03.1999)

(71) Applicant (for all designated States except US): INVITROGEN CORPORATION [US/US]; 1600 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): DALBY, Brian [GB/US]; 2803 Unicornio Street, Carlsbad, CA 92009 (US). BENNETT, Robert, P. [US/US]; 1269 Rainbow Ridge Lane, Encinitas, CA 92024 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

(88) Date of publication of the international search report:  
18 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/58488 A3

(54) Title: DELIVERY OF FUNCTIONAL PROTEIN SEQUENCES BY TRANSLOCATING POLYPEPTIDES

(57) Abstract: The invention provides methods for modulating a cellular process by contacting a cell in culture with a cell process-modifying molecule attached to a translocating polypeptide. For example, in one embodiment, a cell in culture is transfected with a target gene by contacting the cell in culture with a polynucleotide (that contains the target gene) attached to a translocating polypeptide. In another embodiment, expression of a target gene product in a cell in culture that contains a target gene under control of one or more regulatory elements is modulated by contacting the cell in culture with one or more regulatory agents attached to a translocating polypeptide. The one or more regulatory agents are translocated into the cell in culture and interact therein with the one or more regulatory elements to modulate expression of the target gene product by the cell.

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 00/08571

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 C12N15/87 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INVITROGEN: "Voyager(TM) - The power of Translocation" INVITROGEN CATALOGUE, XX, XX, vol. 6, no. 1, February 1999 (1999-02), page 6 XP002140132 the whole document ---	1-50
X	WO 98 32866 A (HARE PETER FRANCIS JOSEPH O ;MARIE CURIE CANCER CARE (GB); ELLIOTT) 30 July 1998 (1998-07-30) cited in the application the whole document ---	1-48
X	WO 97 05265 A (HARE PETER FRANCIS JOSEPH O ;ELLIOTT GILLIAN DAPHNE (GB)) 13 February 1997 (1997-02-13) cited in the application the whole document ---	1-48
	-/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

6 October 2000

Date of mailing of the international search report

23/10/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Niemann, F

**INTERNATIONAL SEARCH REPORT**

International Application No

PCT/US 00/08571

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELLIOTT G ET AL: "Intercellular trafficking of VP22 -GFP fusion proteins" GENE THERAPY, GB, MACMILLAN PRESS LTD., BASINGSTOKE, vol. 6, no. 1, January 1999 (1999-01), pages 149-151, XP002119414 ISSN: 0969-7128 the whole document ---	1-48
X	MURPHY A L ET AL: "Catch VP22: the hitch-hiker's ride to gene therapy?" GENE THERAPY, GB, MACMILLAN PRESS LTD., BASINGSTOKE, vol. 6, no. 1, January 1999 (1999-01), pages 4-5, XP002119415 ISSN: 0969-7128 the whole document ---	1-48
X	WO 99 11809 A (IMP COLLEGE INNOVATIONS LTD ;CRISANTI ANDREA (GB)) 11 March 1999 (1999-03-11) the whole document ---	1-3, 5-15, 17-48
X	PROCHIANTZ A: "Getting hydrophilic compounds into cells: lessons from homeopeptides" CURRENT OPINION IN NEUROBIOLOGY, GB, LONDON, vol. 6, no. 5, 1 October 1996 (1996-10-01), pages 629-634, XP002087113 ISSN: 0959-4388 the whole document ---	1-3, 5-15, 17-48
X	PROCHIANTZ A: "Peptide nucleic acid smugglers" NATURE BIOTECHNOLOGY, US, NATURE PUBLISHING, vol. 16, 1 September 1998 (1998-09-01), pages 819-820, XP002088768 ISSN: 1087-0156 the whole document ---	1-3, 5-15, 17-48
X	BONFANTI M ET AL: "p21 WAF1-derived peptides linked to an internalization peptide inhibit human cancer cell growth" CANCER RESEARCH, US, AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 57, 15 April 1997 (1997-04-15), pages 1442-1446, XP002087115 ISSN: 0008-5472 the whole document ---	1-3, 5-15, 17-48
		-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/08571

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANGEL U ET AL: "Cell penetrating PNA constructs" JOURNAL OF NEUROCHEMISTRY, US, NEW YORK, NY, vol. 69, no. SUPPL, 20 July 1997 (1997-07-20), page S260 XP002088767 ISSN: 0022-3042 the whole document ---	1-3, 5-15, 17-48
X	WO 99 05302 A (PERKIN ELMER CORP) 4 February 1999 (1999-02-04)  the whole document ---	1-3, 5-15, 17-48
A	FRITZ J D ET AL: "GENE TRANSFER INTO MAMMALIAN CELLS USING HISTONE-CONDENSED PLASMID DNA" HUMAN GENE THERAPY, XX, XX, vol. 7, 1 August 1996 (1996-08-01), pages 1395-1404, XP002058321 ISSN: 1043-0342 cited in the application the whole document ---	8
A	NIIDOME TAKURO ET AL: "Binding of cationic alpha-helical peptides to plasmid DNA and their gene transfer abilities into cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 24, 1997, pages 15307-15312, XP002149406 ISSN: 0021-9258 cited in the application the whole document ---	8
A	ZAITSEV S V ET AL: "H1 and HMG17 extracted from calf thymus nuclei are efficient DNA carriers in gene transfer." GENE THERAPY, vol. 4, no. 6, 1997, pages 586-592, XP000952517 ISSN: 0969-7128 cited in the application the whole document ---	8
A	WEN W ET AL: "IDENTIFICATION OF A SIGNAL FOR RAPID EXPORT OF PROTEINS FROM THE NUCLEUS" CELL, US, CELL PRESS, CAMBRIDGE, MA, vol. 82, 11 August 1995 (1995-08-11), pages 463-473, XP002912310 ISSN: 0092-8674 cited in the application the whole document ---	9,10

-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/08571

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEN XIAOZHUO ET AL: "A SELF-INITIATING EUKARYOTIC TRANSIENT GENE EXPRESSION SYSTEM BASED ON COTRANSFECTON OF BACTERIOPHAGE T7 TNA POLYMERASE AND DNA VECTORS CONTAINING A T7 AUTogene" NUCLEIC ACIDS RESEARCH, GB, OXFORD UNIVERSITY PRESS, SURREY, vol. 22, no. 11, 11 June 1994 (1994-06-11), pages 2114-2120, XP002029322 ISSN: 0305-1048 cited in the application the whole document</p> <p>---</p>	19-22
P,X	<p>WO 99 24559 A (ACTINOVA LTD ;AXCRONA EUGEN JAN KAROL (SE); LEANDERSSON TOMAS BORJ) 20 May 1999 (1999-05-20) the whole document</p> <p>-----</p>	1-3, 5-15, 17-48

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 00/08571

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9832866	A 30-07-1998	AU 5674998 A EP 0961829 A US 6017735 A		18-08-1998 08-12-1999 25-01-2000
WO 9705265	A 13-02-1997	AU 705563 B AU 6623996 A BR 9610058 A CA 2227786 A CN 1208438 A EP 0845043 A JP 11510386 T		27-05-1999 26-02-1997 27-07-1999 13-02-1997 17-02-1999 03-06-1998 14-09-1999
WO 9911809	A 11-03-1999	AU 8877698 A EP 1009847 A		22-03-1999 21-06-2000
WO 9905302	A 04-02-1999	AU 8408098 A EP 0998577 A US 6025140 A		16-02-1999 10-05-2000 15-02-2000
WO 9924559	A 20-05-1999	AU 1045999 A EP 1036167 A		31-05-1999 20-09-2000